



# Genomic Portrait of the Evolution and Epidemic Spread of a Recently Emerged Multidrug-Resistant *Shigella flexneri* Clone in China

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ICDC, China CDC

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# Serotype Shift of *Shigella flexneri* in China

<i>S. flexneri</i> Serotypes	Indonesia (% of Total Serotypes)	Vietnam (% of Total Serotypes)	China (% of Total Serotypes)	Thailand(% of Total Serotypes)
1a	63 (7%)	1 (0%)	104 (34%)	0 (0%)
1b	105 (12%)	7 (2%)	0 (0%)	5 (23%)
1c	103 (12%)	0 (0%)	0 (0%)	0 (0%)
2a	297 (34%)	69 (24%)	84 (28%)	8 (36%)
2b	5 (1%)	103 (37%)	1 (0%)	0 (0%)
3a	142 (16%)	58 (21%)	1 (0%)	1 (5%)
3b	10 (1%)	3 (1%)	0 (0%)	5 (23%)
3c	0 (0%)	0 (0%)	0 (0%)	0 (0%)
4	12 (1%)	11 (4%)	7 (2%)	0 (0%)
4a	53 (6%)	4 (1%)	0 (0%)	1 (5%)
4b	1 (0%)	0 (0%)	0 (0%)	0 (0%)
4x	11 (1%)	0 (0%)	0 (0%)	0 (0%)
5a	2 (0%)	0 (0%)	1 (0%)	0 (0%)
6	38 (4%)	12 (4%)	0 (0%)	0 (0%)
x	2 (0%)	0 (0%)	100 (33%)	0 (0%)
y	5 (1%)	2 (1%)	5 (2%)	0 (0%)
Not typed	17 (2%)	12 (4%)	2 (0%)	2 (9%)
Total	866 (100%)	282 (100%)	305 (100%)	22 (100%)

# New Serotype of *S. flexneri* emerged in 2000 in China

Commercial diagnostic antibody				
Serotype	Type 4	Group 3,4	Group 6	Group 7,8
4c	+	-	-	+
monoclonal antibodies				
	Type 4-specific MASF IV-2		group antigen-specific MASF IV-1	
4X	-		+	

*Carlin, N. I., and A. A. Lindberg. IAI 1987*

# SEROTYPE CONVERTING PHAGE Sfx

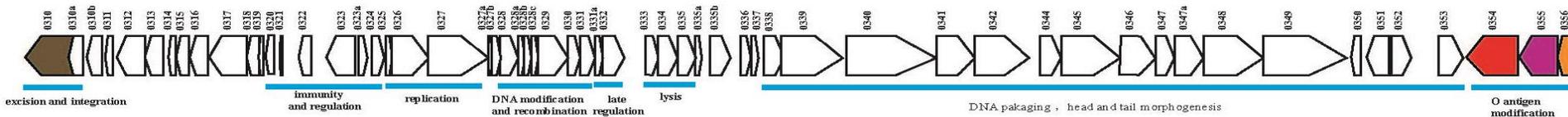
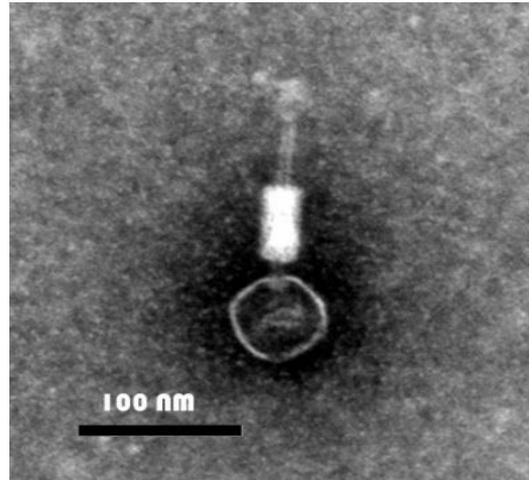
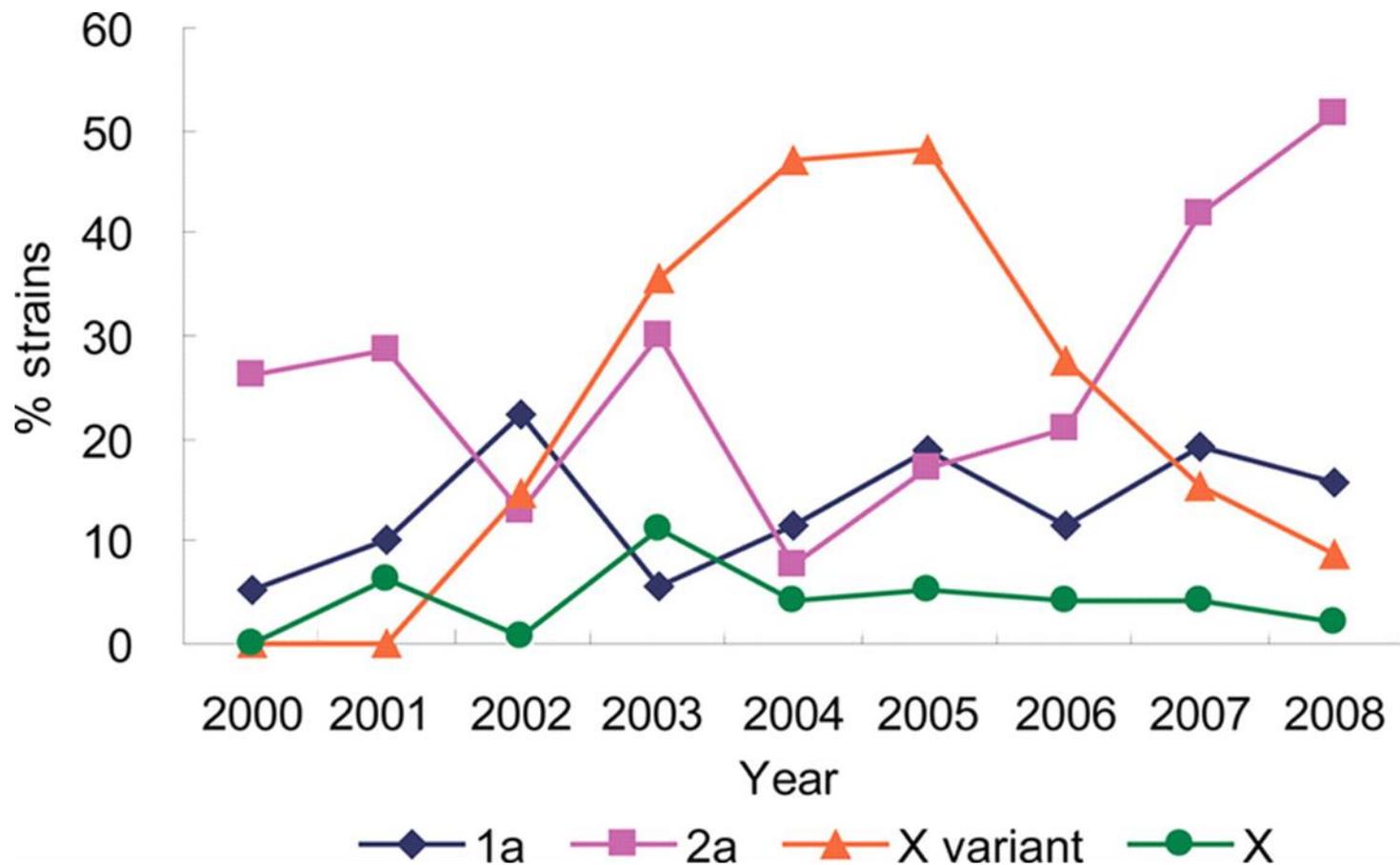
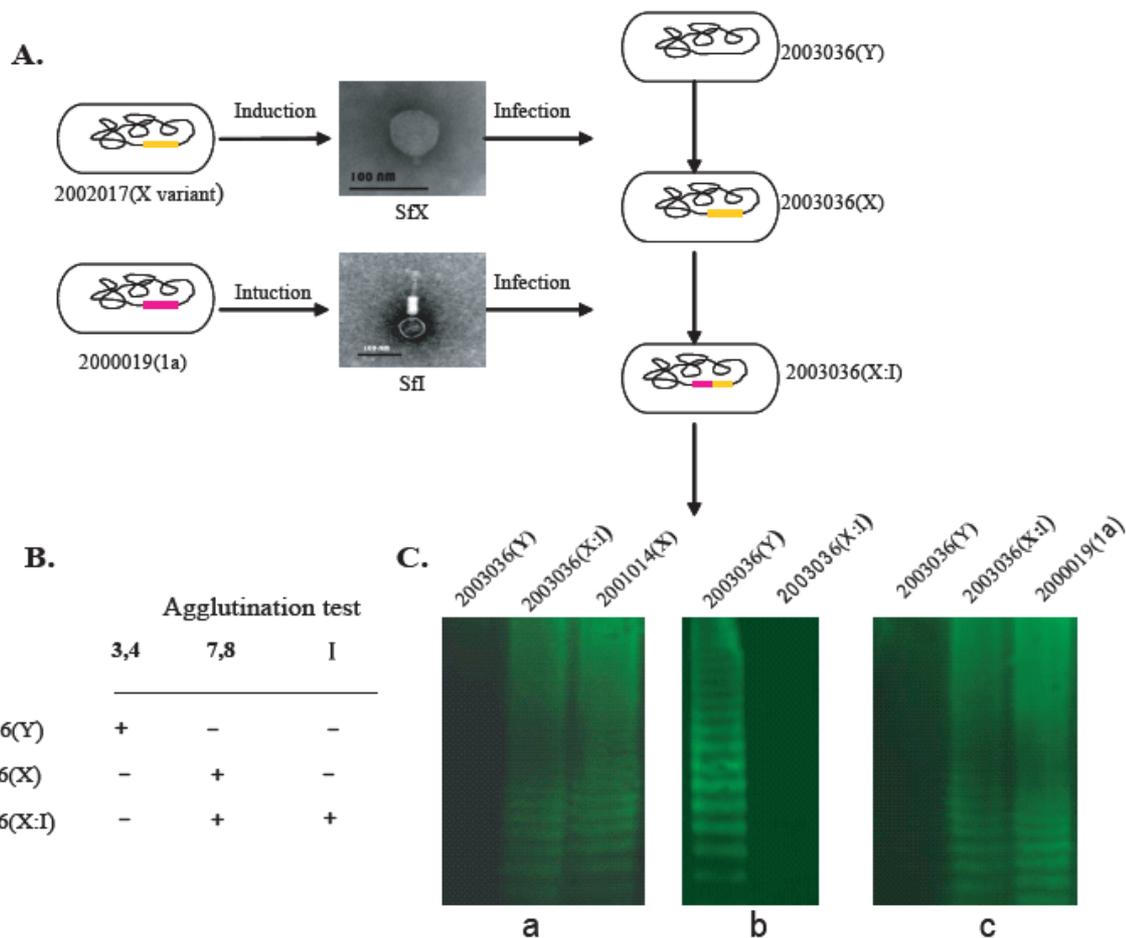


Figure S3 Genetic map of the SHI-O island (Sfx phage) of 2002017. The ORFs are drawn to scale with direction of transcription shown. Locations of the different ORFs are marked with locus tags above the genetic map. Functional modules are indicated below the genetic map and are based on sequence homology to lambdoid phages<sup>1, 2</sup> and other *Sh. flexneri* serotype conversion phages<sup>3, 4</sup>. The gtr genes are shown in colour.

# Emergence of *Shigella flexneri* serotype X variant in China



# Construction of a novel *S. flexneri* serotype 1d using serotype-converting bacteriophage SfX and Sfl

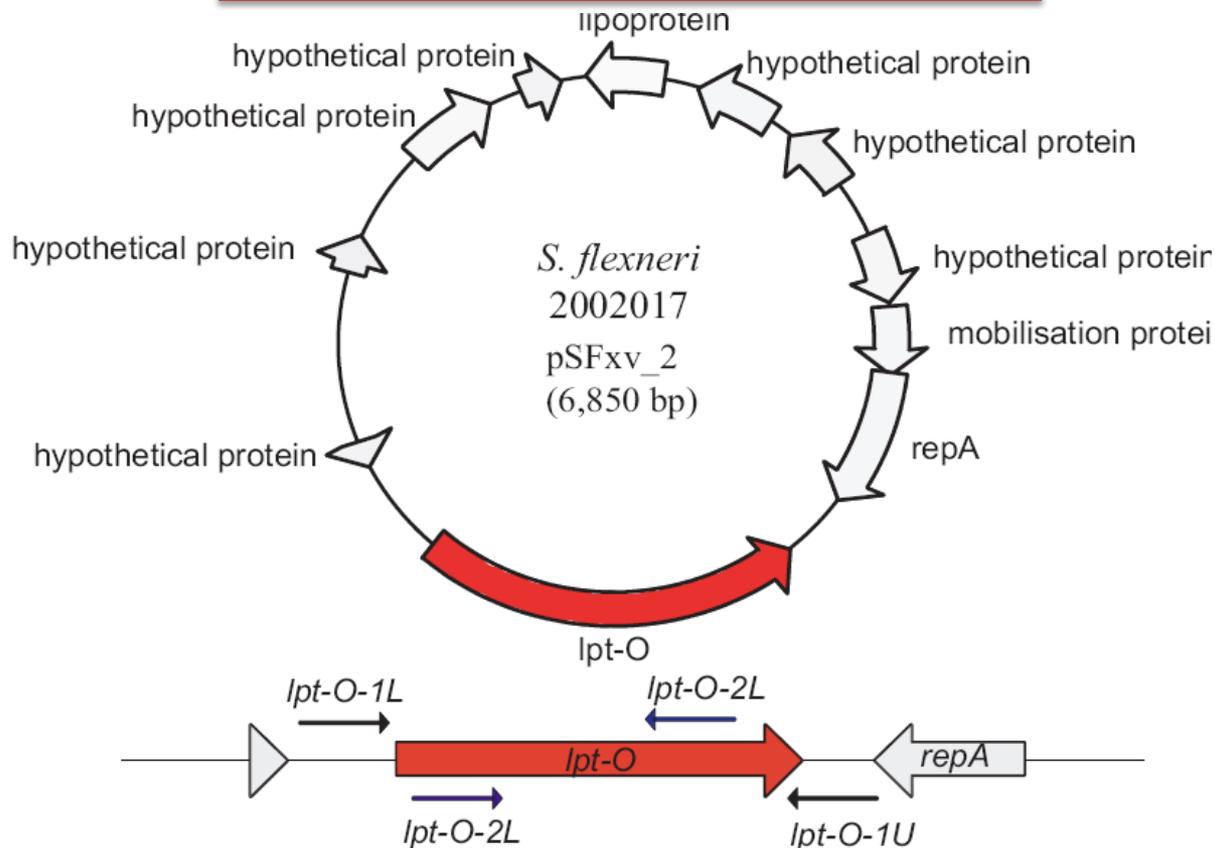


# SEROTYPE CONVERTING PLASMID

encoding LPS phosphoethanolamine transferase *lptO*

Addition of phosphoethanolamine (PEtN) to position 3 of either RhIII or RhII, or both, which confers the host with the MASF IV-1 (E1037) epitope called “variant”

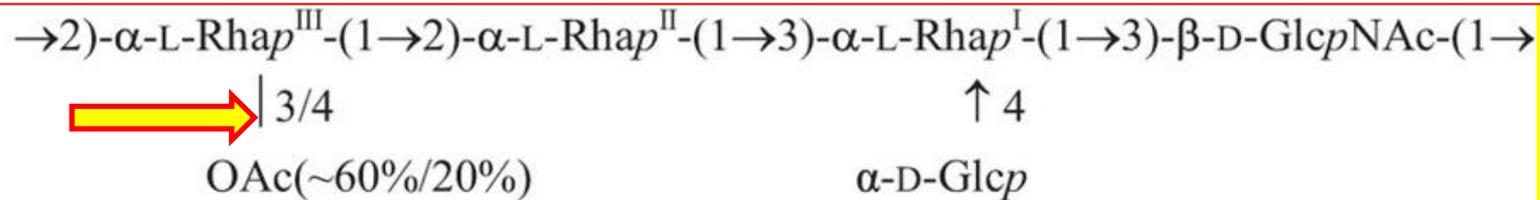
group antigen-specific MASF IV-1 ???



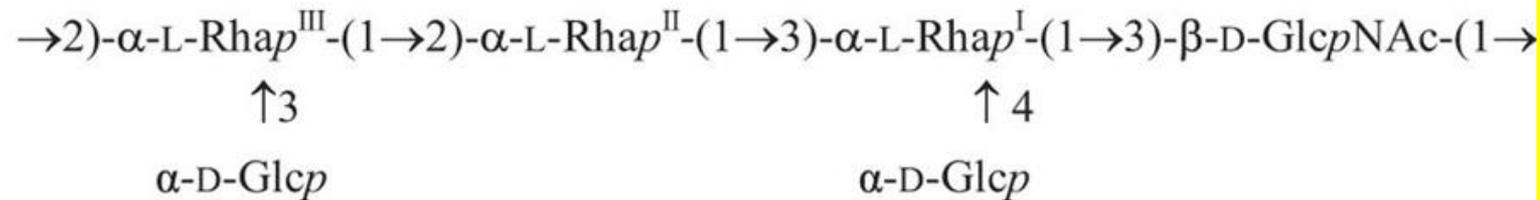
# A new group 9 antigen was recently identified

**3- and 4-O-Acetylated Rhamnose III of Lipopolysaccharide**  
mediated by *oacB* on transposon-like element

51251\_pSQZ4



51251 (2b)



On a transposon-like element

# Group antigen 9 is common *in S.flexneri*

serotype 1a, 1b, 2a, 5a, Y, and 6

Serotype	No. of strains tested	No. (%) of <i>oacB</i> -positive strains	No. (%) of strains that cross-react with grouping antiserum 9
1a	107	102 (95.33)	102 (95.33)
1b	25	25 (100)	25 (100)
1c	3	0	0
1d	14	0	0
2a	169	163 (96.45)	160 (94.75)
2b	61	21 (34.43)	0
3a	18	0	0
3b	4	0	0
4a	4	0	0
4av	4	0	0
4b	4	0	0
5a	14	9 (64.29)	9 (64.29)
5b	5	0	0
X	50	3 (6.00)	0
Xv	126	1 (0.79)	0
Y	39	25 (64.10)	24 (61.54)
Yv	20	0	0
6	59	0	59 (100)
7b	4	0	0
<b>Total</b>	<b>730</b>	<b>349 (47.80)</b>	<b>382 (52.33)</b>

# A new group antigen 10 was recently identified

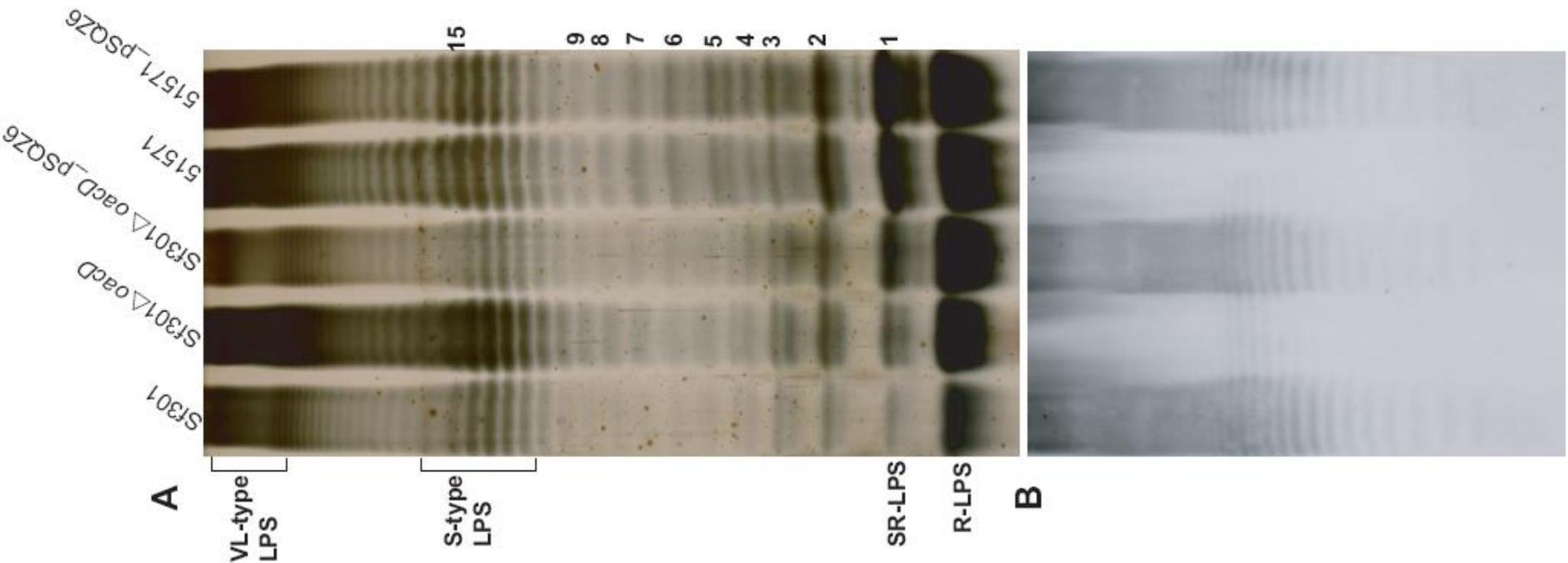
*oacD*-mediated 6-O-acetylation on GlcNAc

On a serotype-converting phage SflI

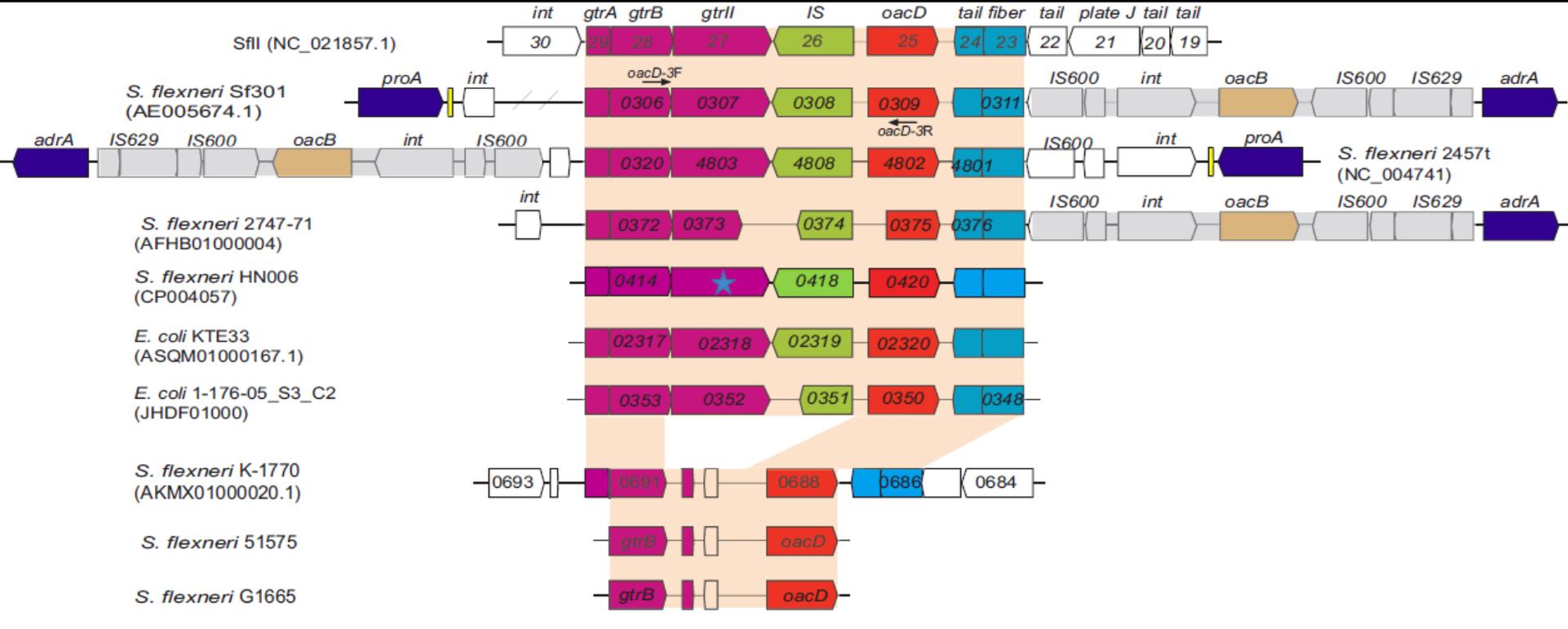
*oacA* for the 2-O-acetylation of RhaI

*oacB* and *oacC* for the 3/4-O-acetylation of RhaIII

*oacD* for 6-O-acetylation of GlcNAc



# *oacD* carried on serotype-converting bacteriophage SfII

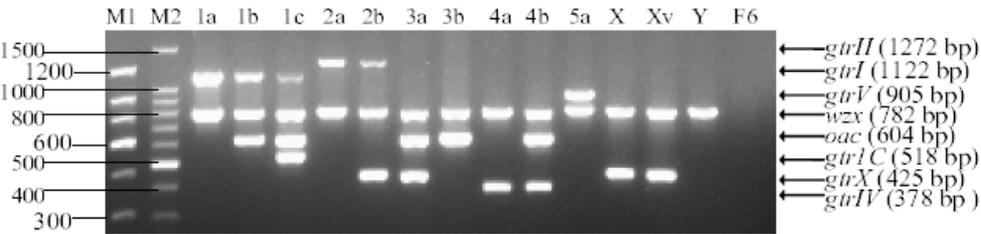


the *oacD* always co-exists with the *gtr* locus in serotype 2 strains and is also present in some other serotypes carrying a cryptic SfII prophage with a **defective *gtr* locus**

# Group antigen 10 is common in *S.flexneri*

Serotype	Number of strains tested	Number of <i>oacD</i> -positive strains	Number of antiserum 10-reactive strains
1a	76	0	0
1b	22	0	0
1c	1	0	0
1d	5	0	0
2a	154	154	154
2b <sup>a</sup>	29	29	23
3a <sup>b</sup>	7	2	2
3b	2	0	0
4a	3	0	0
4av	3	0	0
4b	3	0	0
5a	2	0	0
X <sup>c</sup>	39	1	1
Xv <sup>d</sup>	189	1	0
Y <sup>e</sup>	37	14	13
Yv <sup>f</sup>	21	13	13
6	76	0	0
7b	2	0	0
Total	672	214	206

# Molecular Serotyping of *S. flexneri*



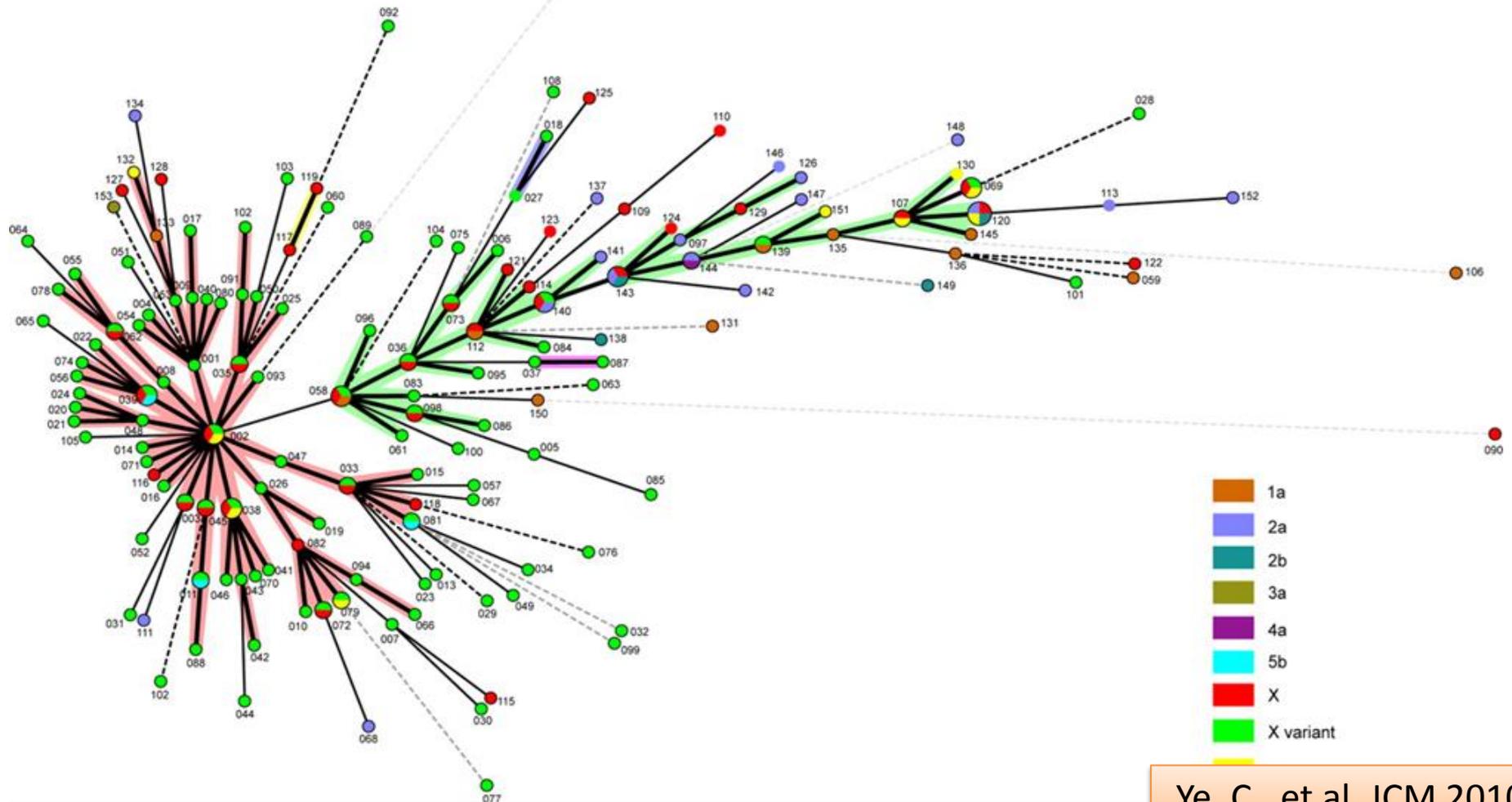
**Sequence screening first, confirmed by serological agglutination**

TABLE 1 Serotype characteristics of *S. flexneri* reference strains by agglutination and multiplex PCR

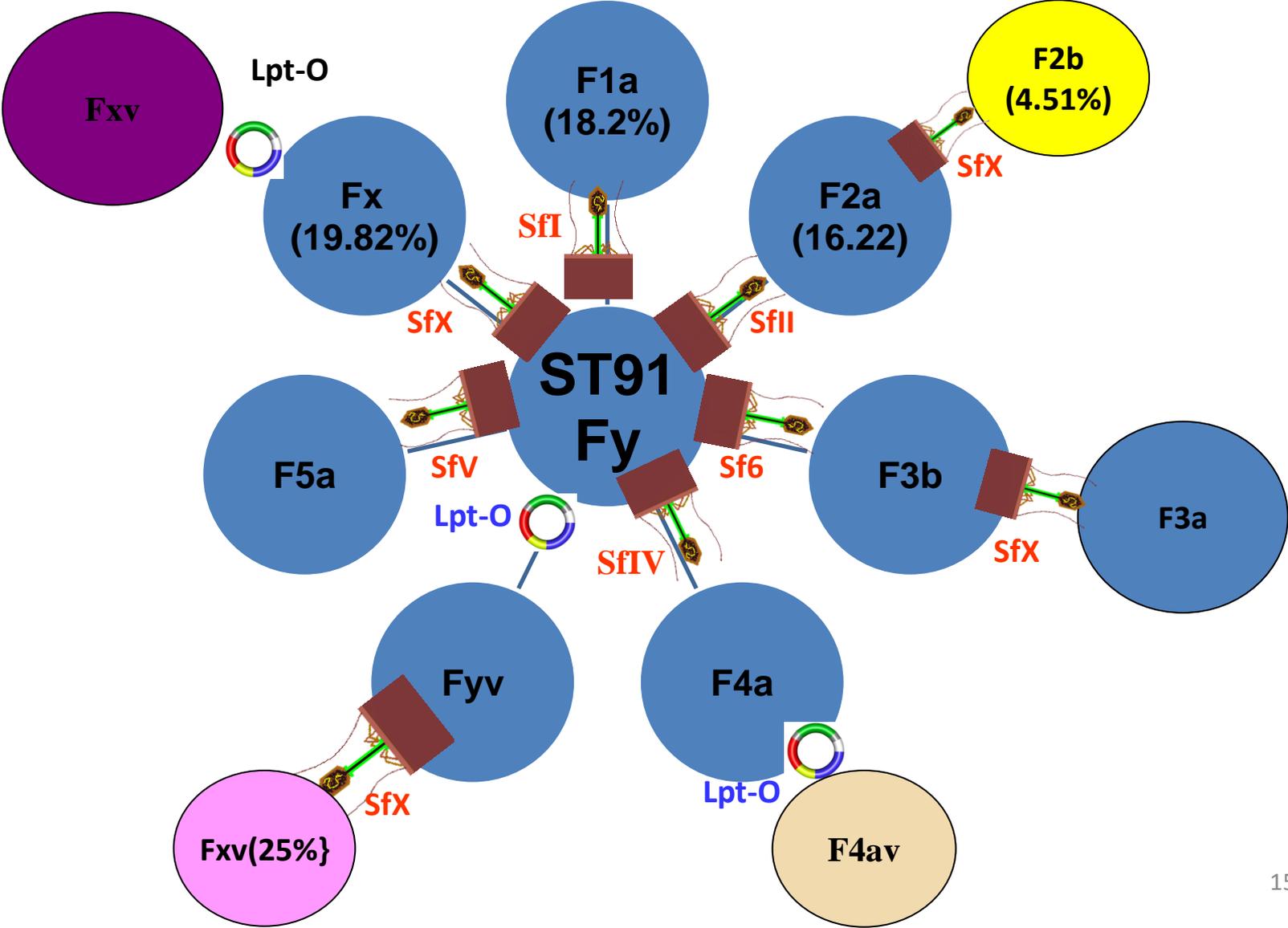
Strain	Serotype	Agglutination					Multiplex PCR											
		Typing				MASF 1c	Grouping			<i>wzx</i>	<i>gtrI</i>	<i>gtrC</i>	<i>gtrII</i>	<i>oac</i>	<i>gtrIV</i>	<i>gtrV</i>	<i>gtrX</i>	
		I	II	III	IV		V	VI	3;4									6
2000019	1a	+	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-
1997020	1b	+	-	-	-	-	-	+	+	-	-	+	+	-	-	+	-	-
06HN081 <sup>#</sup>	-	-	-	-	-	-	-	-	+	-	+	+	+	-	+	-	-	-
301	2a	-	+	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-
NCTC4	2b	-	+	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+
03HL12	3a	-	-	+	-	-	-	-	+	+	-	+	-	-	+	-	-	+
2002110	3b	-	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	-
NCTC9725	4a	-	-	-	+	-	-	+	-	-	-	+	-	-	-	+	-	-
NCTC9726	4b	-	-	-	+	-	-	-	+	-	-	+	-	-	+	+	-	-
51247	5a	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	+	-
2003036	Y	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-
2001014	X	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+
2002017	Xv	-	-	-	+	-	-	-	-	+	-	+	-	-	-	-	-	+
2000007	F6	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

# Minimum spanning tree of *S. flexneri* isolates based on PFGE data

A total of 655 isolates obtained from Henan and 10 other provinces of China were analyzed and divided into 154 pulse types



# Hypothesis for serotype conversion of *Shigella flexneri*



We aimed to elucidate the temporal and geographical dynamics of *S. flexneri* epidemics in China across a 10-year time span. We sequenced 59 *S. flexneri* isolates of 14 serotypes (serotypes 1 to 4, Y, Yv, X, and Xv).

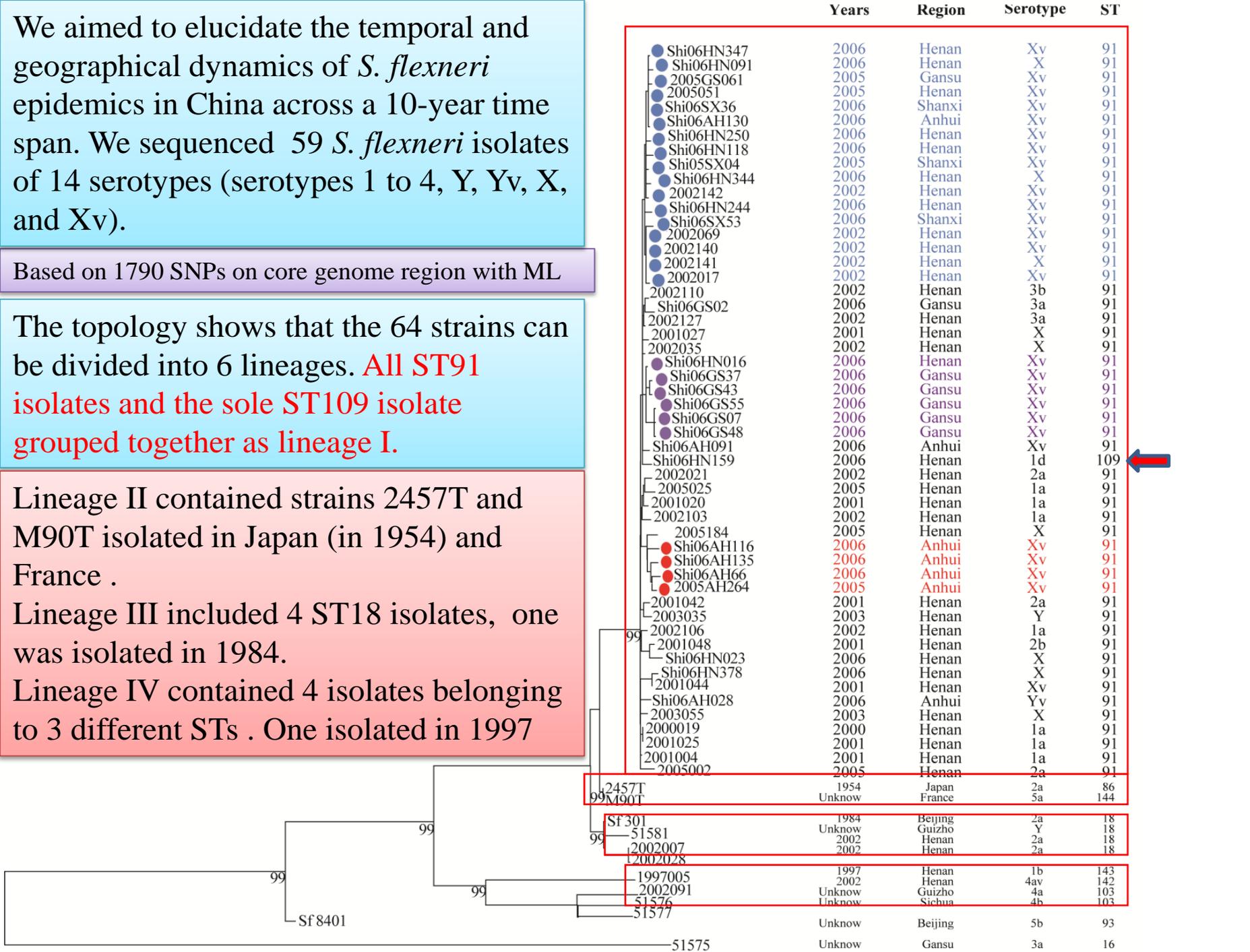
Based on 1790 SNPs on core genome region with ML

The topology shows that the 64 strains can be divided into 6 lineages. All ST91 isolates and the sole ST109 isolate grouped together as lineage I.

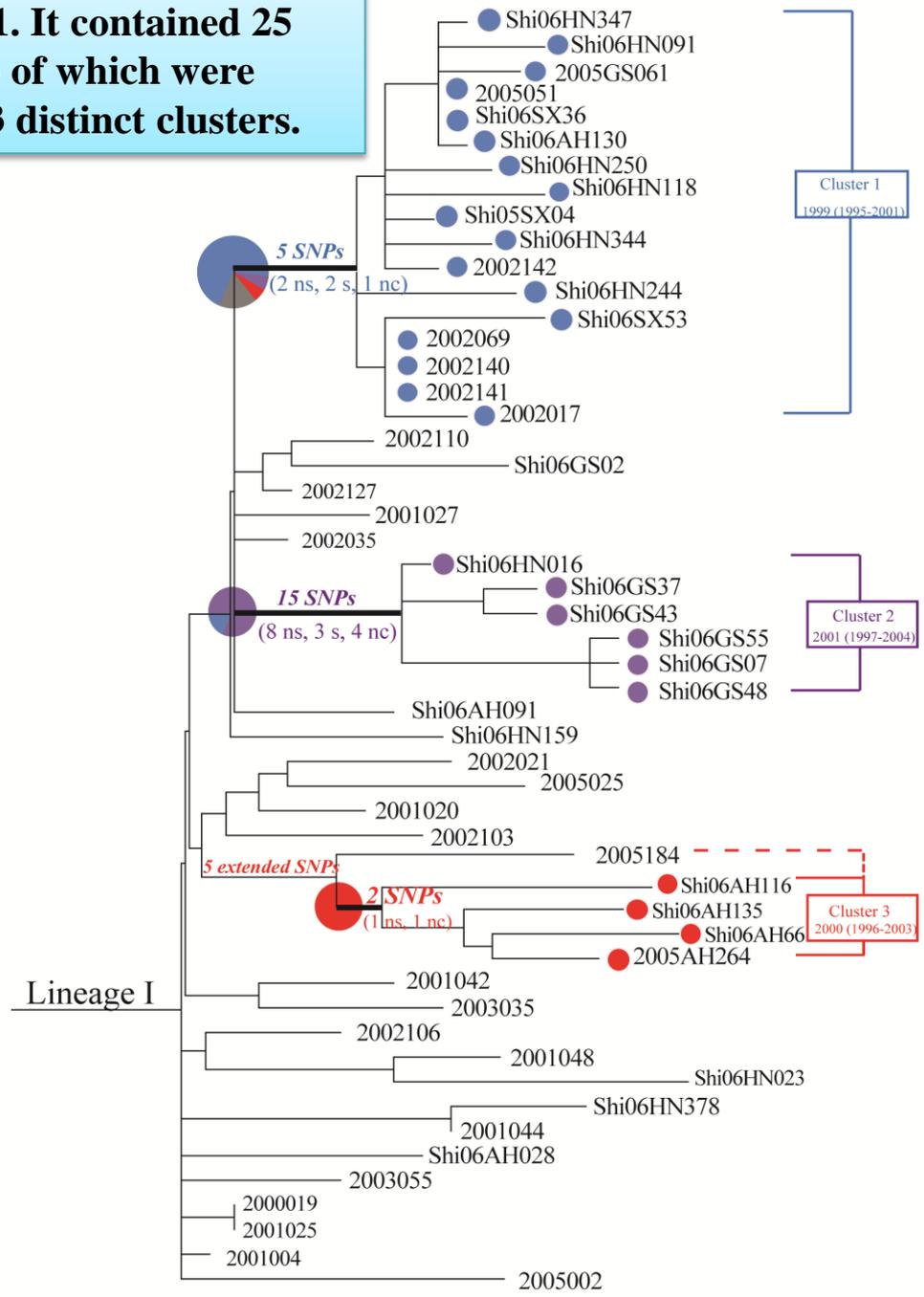
Lineage II contained strains 2457T and M90T isolated in Japan (in 1954) and France .

Lineage III included 4 ST18 isolates, one was isolated in 1984.

Lineage IV contained 4 isolates belonging to 3 different STs . One isolated in 1997



**Lineage I exclusively contained epidemic ST91. It contained 25 Xv isolates, 23 of which were grouped into 3 distinct clusters.**



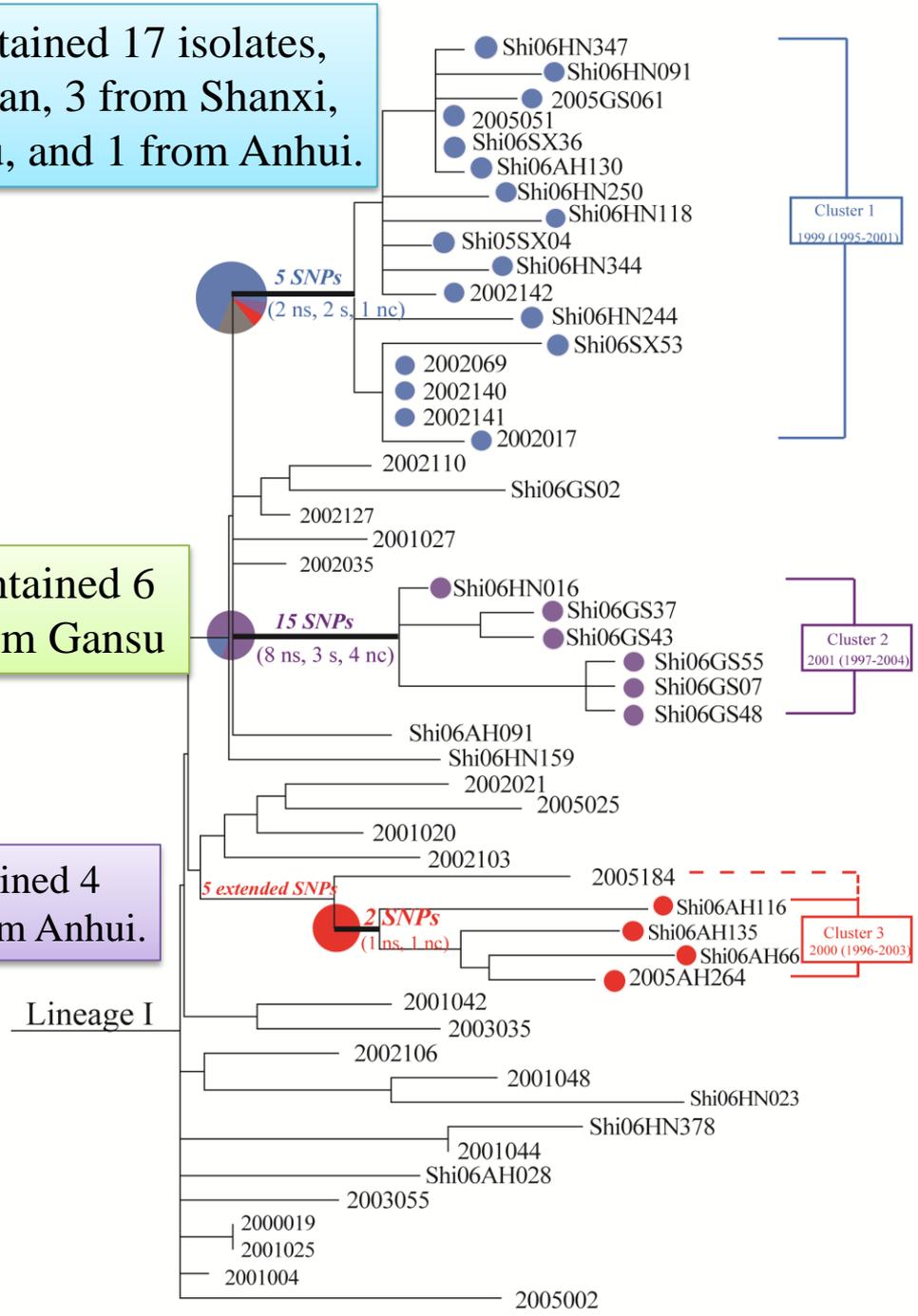
years	Region	Serotype	ST
2006	Henan	Xv	91
2006	Henan	X	91
2005	Gansu	Xv	91
2005	Henan	Xv	91
2006	Shanxi	Xv	91
2006	Anhui	Xv	91
2006	Henan	Xv	91
2006	Henan	Xv	91
2006	Henan	Xv	91
1999 (1995-2001)	Shanxi	Xv	91
2006	Henan	X	91
2002	Henan	Xv	91
2006	Henan	Xv	91
2006	Shanxi	Xv	91
2002	Henan	Xv	91
2002	Henan	Xv	91
2002	Henan	X	91
2002	Henan	Xv	91
2002	Henan	3b	91
2006	Gansu	3a	91
2002	Henan	3a	91
2001	Henan	X	91
2002	Henan	X	91
2006	Henan	Xv	91
2006	Gansu	Xv	91
2006	Gansu	Xv	91
2006	Gansu	Xv	91
2006	Gansu	Xv	91
2006	Anhui	Xv	91
2006	Henan	1d	109
2002	Henan	2a	91
2005	Henan	1a	91
2001	Henan	1a	91
2002	Henan	1a	91
2005	Henan	X	91
2006	Anhui	Xv	91
2006	Anhui	Xv	91
2006	Anhui	Xv	91
2005	Anhui	Xv	91
2001	Henan	2a	91
2003	Henan	Y	91
2002	Henan	1a	91
2001	Henan	2b	91
2006	Henan	X	91
2006	Henan	X	91
2001	Henan	Xv	91
2006	Anhui	Yv	91
2003	Henan	X	91
2000	Henan	1a	91
2001	Henan	1a	91
2001	Henan	1a	91
2005	Henan	2a	91



Cluster 1 contained 17 isolates, 12 from Henan, 3 from Shanxi, 1 from Gansu, and 1 from Anhui.

Cluster 2 contained 6 isolates, 5 from Gansu

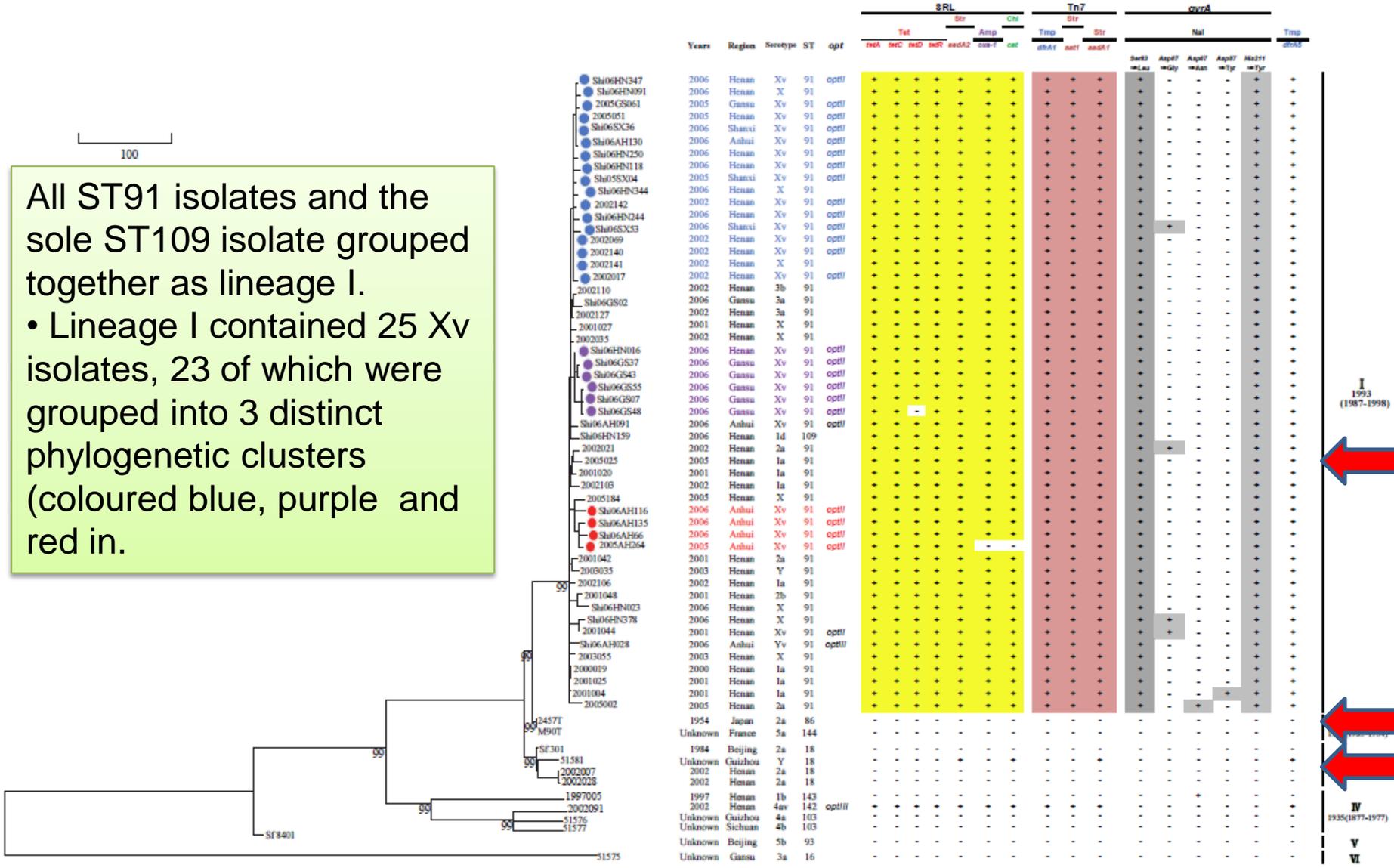
Cluster 3 contained 4 isolates, all from Anhui.



years	Region	Serotype	ST
2006	Henan	Xv	91
2006	Henan	X	91
2005	Gansu	Xv	91
2005	Henan	Xv	91
2006	Shanxi	Xv	91
2006	Anhui	Xv	91
2006	Henan	Xv	91
2006	Henan	Xv	91
2006	Henan	Xv	91
1999 (1995-2001)	Shanxi	Xv	91
2005	Shanxi	Xv	91
2006	Henan	X	91
2002	Henan	Xv	91
2006	Henan	Xv	91
2006	Shanxi	Xv	91
2002	Henan	Xv	91
2002	Henan	Xv	91
2002	Henan	X	91
2002	Henan	Xv	91
2002	Henan	3b	91
2006	Gansu	3a	91
2002	Henan	3a	91
2001	Henan	X	91
2002	Henan	X	91
2006	Henan	Xv	91
2006	Gansu	Xv	91
2006	Gansu	Xv	91
2006	Gansu	Xv	91
2006	Gansu	Xv	91
2006	Gansu	Xv	91
2006	Anhui	Xv	91
2006	Henan	1d	109
2002	Henan	2a	91
2005	Henan	1a	91
2001	Henan	1a	91
2002	Henan	1a	91
2005	Henan	X	91
2006	Anhui	Xv	91
2006	Anhui	Xv	91
2006	Anhui	Xv	91
2005	Anhui	Xv	91
2001	Henan	2a	91
2003	Henan	Y	91
2002	Henan	1a	91
2001	Henan	2b	91
2006	Henan	X	91
2006	Henan	X	91
2001	Henan	Xv	91
2006	Anhui	Yv	91
2003	Henan	X	91
2000	Henan	1a	91
2001	Henan	1a	91
2001	Henan	1a	91
2005	Henan	2a	91

All ST91 isolates and the sole ST109 isolate grouped together as lineage I.

- Lineage I contained 25 Xv isolates, 23 of which were grouped into 3 distinct phylogenetic clusters (coloured blue, purple and red in).



I  
1993  
(1987-1998)

IV  
1935(1877-1977)

V

VI



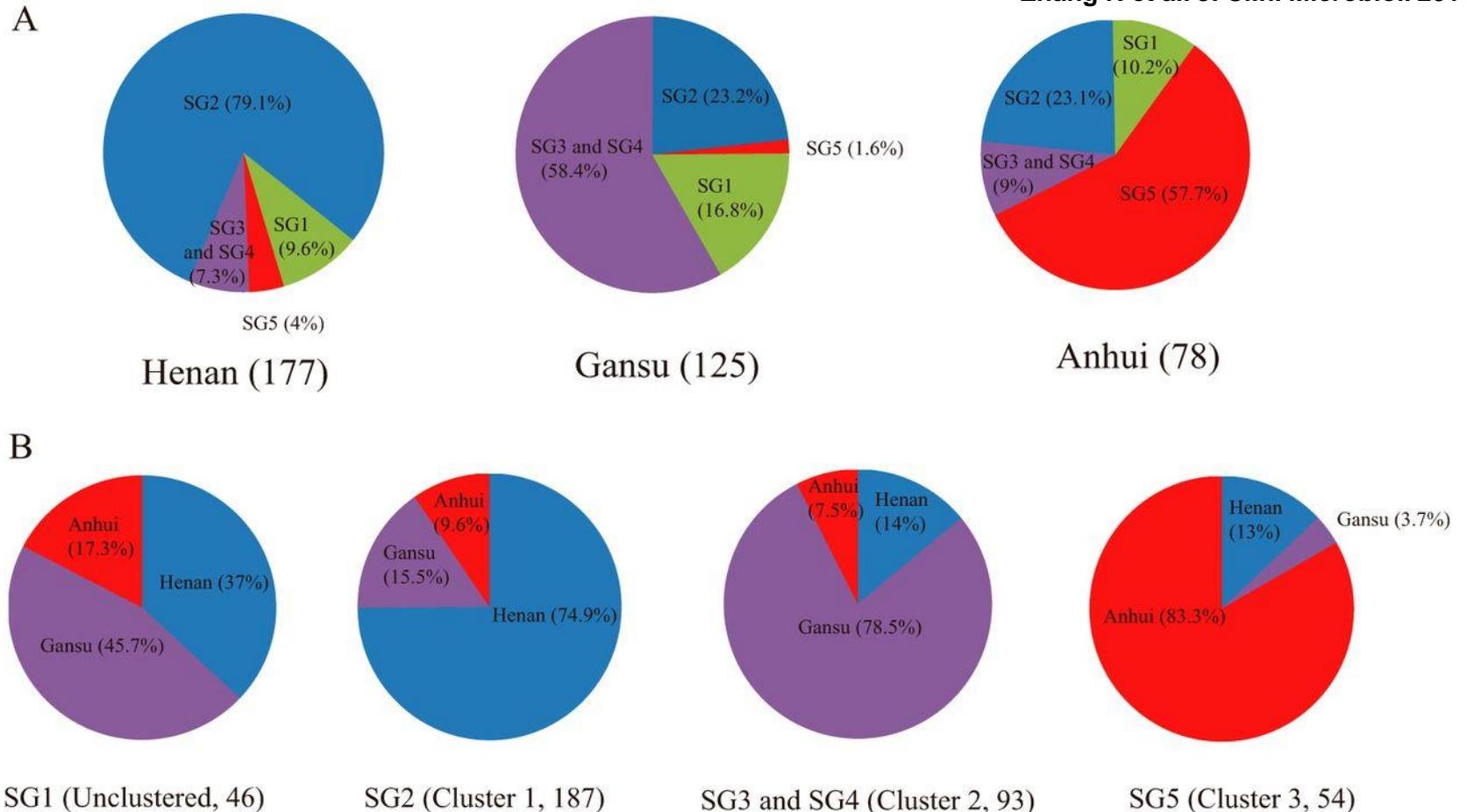
**Genomic relationships of 64 *S. flexneri* isolates.** The tree was based on 1790 SNPs constructed by the maximum likelihood method. The *Shigella* resistance locus (SRL) island, Tn7, *gyrA* mutations and *dfra5* were on right. The 3 serotype X clusters 1, 2 and 3 were coloured blue, purple and red respectively. Lineages are indicated by roman numerals. Lineage age was inferred using BEAST.

# Geographic distribution of the SNP genotypes of the 380 *S. flexneri* isolates in China

(A) Frequencies of the 5 SGs in different provinces

(B) Geographic distribution of different SGs

Zhang N et al. J. Clin. Microbiol. 2014



The 18 SNPs divided the 380 isolates into 5 SNP genotypes (SGs). SG1 contained 46 isolates, including 17 from Henan, 21 from Gansu, and 8 from Anhui. SG1 grouped together all isolates that did not belong to any of the 3 clusters. SG2 and SG5 corresponded to genome clusters 1 and 3, respectively. Cluster 2 was divided into SG3 and SG4

# NEWS & ANALYSIS

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## GENOME WATCH

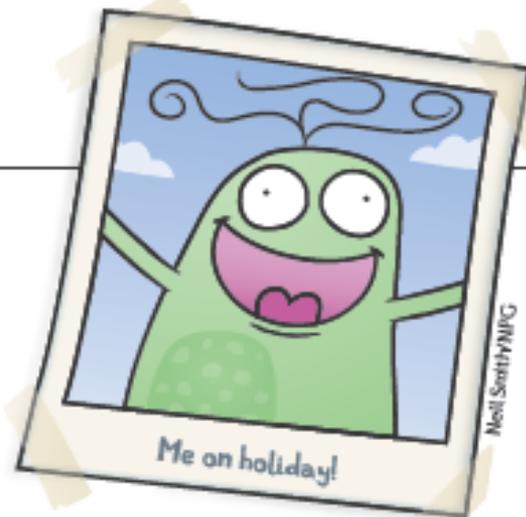
### TB or not TB? Genomic portraits provide answers

Kate S. Baker and Matthew J. Ellington

This month's Genome Watch highlights the application of bacterial whole-genome sequencing in public health microbiology and epidemiological profiling.

and, in some cases, occurred in combination with epistatic mutations that compensated for the fitness costs of resistance. For example, mutations in *rpoA*, *rpoB* and *rpoC* that compensated for resistance-conferring *rpoB*

epidemic bacterial strains. These studies also show that transmission occurs at household and regional levels and that WGS is useful for providing details of antimicrobial resistance genes and compensatory mutations. Both



My Team:

Qiangzheng Sun, Nan Zhang, Jianping Wang, Changyun Ye ,Ruiting Lan

